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ORIGINAL INVESTIGATION

Role of serotonin 5-HT_{2A} and 5-HT_{2C} receptors on brain stimulation reward and the reward-facilitating effect of cocaine

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Abstract
Rationale The serotonin 5-HT_{2A} and 5-HT_{2C} receptors, which are found in abundance in the mesolimbocortical dopaminergic system, appear to modulate the behavioral effects of cocaine.
Objectives The present series of studies set out to investigate the role of 5-HT_{2A} and 5-HT_{2C} receptors on brain reward and on the reward-facilitating effect of cocaine and localize the neural substrates within the mesolimbocortical dopaminergic system that are responsible for these effects.
Methods Male Sprague-Dawley rats were implanted with stimulating electrodes and bilateral cannulae for the experiments involving microinjections and were trained to respond to electrical stimulation. In the first study, we examined the effects of systemic administration of selective 5-HT_{2A} and 5-HT_{2C} receptor agonists (TCB-2 and WAY-161503) and antagonists (R-96544 and SB-242084) on intracranial self-stimulation (ICSS). In the second study, we examined the effectiveness of TCB-2, WAY-161503, R-96544, and SB-242084 in blocking the reward-facilitating effect of cocaine. In the third study, we examined the effects of intra-medial prefrontal cortex (mPFC), intra-nucleus accumbens (NAC), and intra-ventral tegmental area (VTA) injection of WAY-161503 on the reward-facilitating effect of cocaine.
Results Acute systemic administration of TCB-2 and WAY-161503 increased ICSS threshold. Systemic WAY-161503 attenuated the reward-facilitating effect of cocaine. This effect was reversed by pretreatment with SB-242084. Intracranial microinjections of WAY-161503 into the mPFC and the NAC shell/core, but not the VTA, attenuated the reward-facilitating effect of cocaine.
Conclusion These data indicate that 5-HT_{2C} receptors within the mPFC and the NAC modulate the reinforcing effects of cocaine and provide evidence that 5-HT_{2C} receptor agonists could be a possible drug discovery target for the treatment of psychostimulant addiction.

Keywords Intracranial self-stimulation · Psychostimulants · 5-Hydroxytryptamine · Mesolimbocortical system · Reward · Ventral tegmental area · Nucleus accumbens · Medial prefrontal cortex

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Introduction

The mesolimbocortical dopaminergic system originating from the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAC) and the medial prefrontal cortex (mPFC) plays an important role in mediating the behavioral effects of addictive substances, such as cocaine (Di Chiara and Bassareo 2007; Pierce and Kumaresan 2006; Thomas et al. 2008; Wise 1998). Despite our understanding of the role of dopamine (DA) in the behavioral effects of cocaine, the utility of dopaminergic agents as effective pharmacotherapeutic medications in cocaine addiction has been limited (Karila et al. 2008; Platt et al. 2002; Xi and Gardner 2008). According to several studies, serotonin may represent another valuable target in the search for an effective pharmacotherapy of cocaine addiction (Bubar and Cunningham 2008; Filip et al. 2005; Muller et al. 2007). Serotoninergic neurons provide a dense innervation of terminals to the VTA, NAC, and mPFC (Halliday and Tork 1989), and serotonin can influence cocaine's action through

modulation of the mesolimbocortical dopaminergic system by direct effects on its receptors. Among the various 5-HT receptor subtypes, several studies point to the potential involvement of the 5-HT_2 receptor family, and especially the 5-HT_{2A} and the 5-HT_{2C} receptors, in the modulation of the behavioral effects of cocaine (Bubar and Cunningham 2006; Muller and Huston 2006). Indeed, both the 5-HT_{2A} and the 5-HT_{2C} receptors have repeatedly been shown to be expressed throughout the brain (Abramowski et al. 1995; Bubar and Cunningham 2007; Hoyer et al. 1986; Liu et al. 2007; Lopez-Gimenez et al. 1997; Nocjar et al. 2002; Sharma et al. 1997) and appear to co-exist in several brain regions of the mesolimbocortical dopaminergic system, such as the VTA, the NAC, and the mPFC (Pompeiano et al. 1994).

Due to the availability of pharmacologically selective serotoninergic compounds, several studies have investigated the relative contribution of 5-HT_{2A} and 5-HT_{2C} receptors in the behavioral effects of cocaine. Thus, acute systemic injections of 5-HT_{2A} receptor antagonists and 5-HT_{2C} receptor agonists block, while acute systemic injections of 5-HT_{2A} receptor agonists and 5-HT_{2C} receptor antagonists enhance the hyperlocomotion induced by cocaine and the discriminative stimulus effects of cocaine (Filip et al. 2004, 2006; Fletcher et al. 2002a, b; McCreary and Cunningham 1999). Differential influences of the 5-HT_{2A} and 5-HT_{2C} receptors on the reinforcing/rewarding properties of cocaine have been also revealed using the self-administration model. Thus, selective or preferential 5-HT_{2A} receptor antagonists did not alter cocaine self-administration (Fletcher et al. 2002a). However, 5-HT_{2C} receptor antagonists increase, whereas 5-HT_{2C} receptor agonists reduce the rate of cocaine self-administration (Fletcher et al. 2004; Fletcher et al. 2002a; Grottick et al. 2000). In addition, studies using the cocaine-induced conditioned hyperactivity paradigm have shown that 5-HT_{2C} receptor stimulation reduced, while 5-HT_{2C} receptor blockade enhanced cocaine-induced conditioned hyperactivity (Liu and Cunningham 2006). Finally, 5-HT_{2A} receptor blockade or 5-HT_{2C} receptor stimulation attenuated cocaine-induced reinstatement of seeking behavior (Fletcher et al. 2002a; Grottick et al. 2000; Neisewander and Acosta 2007).

An animal model that may be particularly useful in the study of the reward-related properties of addictive drugs is the intracranial self-stimulation (ICSS) paradigm (Carlezon and Chartoff 2007). This behavioral paradigm is based on the discovery by Olds and Milner that rats will repeatedly press a lever to stimulate specific components of the brain reward circuit (Olds and Milner 1954). A number of studies have confirmed the validity of this technique for the assessment of both the rewarding and the anhedonic effects of drugs or other manipulations (Benaliouad et al. 2007; Gallo et al. 2010; Kornetsky 1985; Markou and Koob 1991; Morissette and Boye 2008; Paterson et al. 2000; Romeas et al. 2009; Slattery et al. 2007; Wise 1996). Most drugs of abuse are able to lower the brain stimulation reward threshold, an effect which supports the notion that they activate the same substrate with electrical stimulation in a synergistic manner (Wise 1996, 1998).

The first purpose of the present study was to explore the contribution of 5-HT_{2A} and 5-HT_{2C} receptors to the reinforcing effects of lateral hypothalamic stimulation and to the ability of cocaine to potentiate such stimulation. According to our results, only the 5-HT_{2C} receptor agonist WAY-161503 reduces the reward-facilitating effect of cocaine. Since the effects of local stimulation of 5-HT_{2C} receptors on cocaine-related behaviors differ considerably, depending on the site of injection, the following experiments were designed to provide a more precise description of the sites within the mesolimbocortical dopaminergic system that mediate this effect. To this end, we examined the effect of WAY-161503 microinjected into the VTA, the NAC, or the mPFC on the reward-facilitating effect of cocaine.

Materials and methods

Animals and surgery

Male Sprague-Dawley rats weighing 300–350 g at the time of surgery were used. Before surgery they were housed in groups of three and maintained on a 12-h light–12-h dark cycle (lights on from 0800 to 2000 hours) with free access to food and water. The animals were anesthetized with intramuscular (i.m.) injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Atropine sulfate (0.6 mg/kg, i.m.) was injected to reduce bronchial secretion. The animals were implanted with a monopolar stimulation electrode aimed at the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (2.56 mm posterior to bregma, 1.8 mm lateral from midsaggital suture, and 8.6 mm below the outer flat skull), according to (Paxinos and Watson 2007). The animals which were used for intracranial microinjections were also implanted with guide cannulae positioned bilaterally toward the medial prefrontal cortex (1.8 mm anterior to bregma, 0.6 mm lateral from midsaggital suture, and 3.6 mm below the outer flat skull), the nucleus accumbens core (1.4 mm anterior to bregma, 2.0 mm lateral from midsaggital suture, and 7.5 mm below the outer flat skull), the nucleus accumbens shell (2.2 mm anterior to bregma, 1.0 mm lateral from midsaggital suture, and 7.8 mm below the outer flat skull), or the ventral tegmental area (5.3 mm posterior to bregma, 0.6 mm lateral from midsaggital suture and 7.2 mm below the outer flat skull), according to Paxinos and Watson (2007).

The electrodes were constructed from 0.25-mm stainless steel wire insulated with Epoxylite except for the conically shaped tip. The anode was an uninsulated stainless steel wire connected to an amphenol pin. The guide cannulae were made from modified 23-gauge stainless steel needles. The injectors were made from 30-gauge stainless steel tubing. A 30-gauge copper wire was kept in the guide cannulae between injections. The tip of the dummy cannula was flushed with that of the guide cannula and did not protrude beyond the ventral tip of the guide cannula. The tips of the guide cannulae were located 1.0 mm above the actual injection sites. Five miniature skull screws, the electrode, the anode, and the cannulae were secured to the skull with acrylic dental cement. Following implantation and for the entire duration of the experiments, the animals were housed individually.

Experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Apparatus and procedures for ICSS

After 1 week recovery, the rats were tested for self-stimulation in an operant chamber made of transparent Plexiglas (25-cm wide, 25-cm deep, and 30-cm high). Each chamber was equipped with a stainless steel poke device (lever) 4-cm wide and protruded 2 cm from the left side at a height of 4 cm from the bottom. Each bar-press triggered a constant current stimulator (Med Associates, St. Albans, VT) that delivered a 0.4-s train of rectangular cathodal pulses of constant duration (0.1 ms) and intensity (250 μA) and variable frequency (15–100 Hz, i.e., 6–40 number of pulses/0.4 s). The pulse frequency, i.e., the number of pulses within a train, was progressively increased up to 40 per stimulation train until the subject showed vigorous self-stimulation. During the acquisition phase, the animals were trained to self-stimulate for at least three consecutive days (1 h daily), using stimulation parameters that maintained near maximal bar-pressing rates. After self-stimulation has been acquired and stabilized for a given pulse frequency, rats were trained to self-stimulate using four alternating series of ascending and descending pulse frequencies. The pulse frequency was varied by steps of approximately 0.1 log units. Each frequency was tested within trials of 60 s in duration, followed by an extinction period of 30 s. For each trial, there was an initial "priming" phase during which the animals received three trains of stimulation at the frequency which was available for the specific trial. A rate–frequency determination session lasted about 45 min. One rate–frequency curve was established daily, for 10–14 days, depending on the period when the self-stimulation indices (i.e., curve shift and threshold measure) were stable. The stimulation parameters, ICSS sessions, and data collection were controlled by a computer.

Unequivocally, ICSS behavior has the advantage of not being affected by satiation or dysphoric effects, which are potentially modulated by various drug treatments (Wise 1996). On the other hand, since cocaine seems to affect motor activity/performance capacity in a dose-dependent manner, the use of a reward selective measure, like the curve shift, was requisite. In this method, plotting the responses of the animals against the various pulse frequencies yields a sigmoidal rate–frequency curve as shown in Figs. 7 and 8. Shifts in the lateral position of the curve provide a selective measure of stimulation-produced reward, as elegantly demonstrated by Edmonds and Gallistel (1974), while vertical shifts provide information on motor/performance capacity. Furthermore, this method offers quantitative scaling of drug-induced changes in reward (see, Campbell et al. 1985) which is useful when comparing the effects of different drugs. In other words, the rate–frequency method appears to have reward selectivity that is required in psychopharmacological research (Carlezon and Chartoff 2007; Miliaressis et al. 1986).

Drug testing began for each animal when the function relating bar-pressing rate to pulse frequency (the rate–frequency function) was stable for at least three consecutive days. The criterion for stability was met when the frequency thresholds did not vary by more than 0.1 log units for three consecutive days.

Drugs and drug administration

Cocaine hydrochloride (National Monopoly for Narcotics, Ministry of Health, Greece; 5 mg/kg, intraperitoneally (i.p.) was dissolved in 0.9% saline and injected i.p. at a volume of 1 ml/kg of body weight. The selective 5-HT_{2A} agonist TCB-2, the selective 5-HT_{2A} antagonist R-96544, and the selective 5-HT_{2C} agonist WAY-161503 were dissolved in water for injection and injected at a volume of 1 ml/kg of body weight. The selective 5-HT_{2C} antagonist SB-242084 was also dissolved in water for injection and injected at a volume of 3 ml/kg of body weight. The doses of the serotoninergic compounds and cocaine were selected based on findings from the literature employing these drugs in animal behavioral models (Cryan and Lucki 2000; Fox et al. 2009; Shioda et al. 2008; Vlachou et al. 2003, 2008; Zaniewska et al. 2009).

For intracranial injections, an injection cannula constructed from a 30-gauge stainless steel tubing was inserted into the guide cannula, protruding 1 mm into the target structure, and connected via a polyethylene tubing to a 1-ml microsyringe mounted in microinjection pump with rate 0.5 μl/min. A volume of 0.5 μl of WAY-161503 dissolved in water for injection (or vehicle) was injected bilaterally in

the hand-restrained animal during 1 min. The injection cannula was then left in place for an additional 1 min after the injection, in order to allow sufficient diffusion of the drug.

All animals took part in only one experiment and received all drug treatments of the experiment. The order of testing for various doses of each drug treatment was counterbalanced according to a Latin square design. Furthermore, a 3-day period was allowed between injections; as in previous studies, it has been observed that this period is considered sufficient for the self-stimulation behavior to return to pretreatment levels.

Behavioral studies

Study 1: Effects of systematically administered 5-HT$_{2A}$ and 5-HT$_{2C}$ ligands on brain stimulation reward

In the first study, four groups of animals (n=27) were used to evaluate the effects of the acute administration of TCB-2 (0, 0.03, 0.1, and 0.3 mg/kg, i.p., n=7), R-96544 (0, 0.3, 1, and 3 mg/kg, s.c., n=5), WAY-161503 (0, 0.3, 1, and 3 mg/kg, s.c., n=7), and SB-242084 (0, 0.25, 0.5, and 1 mg/kg, i.p., n=8) on brain stimulation reward. Each drug or vehicle self-stimulation test consisted of a pre-drug and a post-drug rate–frequency function determination (for 45 min each). The injection of the serotoninergic compound was given immediately following the pre-drug rate–frequency function determination. After a post-injection interval of 20 min, the rats were placed in the operant chamber, and the post-drug session began. In a second series of experiments, two groups of rats (n=13) were used in order to examine whether the effects of each selective 5-HT receptor agonist (TCB-2 0.3 mg/kg, i.p., (n=7) and WAY-161503 0.1 mg/kg, s.c. (n=6) could be reversed by pretreatment with their respective 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor antagonist (R-96544 0.3 mg/kg, s.c., and SB-242084 0.5 mg/kg, i.p.).

Study 2: Effects of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor ligands on the reward-facilitating effect of cocaine

In the second study, four groups of animals (n=40) were used to evaluate the effects of the acute administration of TCB-2 (0.1 mg/kg, i.p., n=8), R-96544 (0.3 mg/kg, s.c., n=8), WAY-161503 (0.3 mg/kg, s.c., n=8), and SB-242084 (0.5 mg/kg, i.p., n=8) or vehicle on the reward-facilitating effect of cocaine (5 mg/kg, i.p.). The aforementioned doses for the serotoninergic compounds were selected from the first study as non-efficacious doses on brain stimulation reward. The self-stimulation test consisted of a pre-drug and a post-drug rate–frequency function determination (for 45 min each). Rats were pretreated with the serotoninergic compounds (TCB-2, R-96544, WAY-161503, or SB-242084) or vehicle followed by cocaine or vehicle. The injection of the serotoninergic compound was given immediately following the pre-drug rate–frequency function determination, followed 20 min later by the cocaine injection. After a post-injection interval of 5 min, the rats were placed in the operant chamber, and the post-drug session began. In addition, another group of rats (n=8) received a combination treatment of SB-242084 (0.5 mg/kg, i.p.) and WAY-161503 (0.3 mg/kg, s.c.) followed 20 min later by an injection of cocaine (5 mg/kg, i.p.). The interval between SB and WAY was 20 min. The purpose of this experiment was to examine whether the selective 5-HT2$_A$ antagonist SB-242084 could reverse the action of the 5-HT2$_A$ agonist WAY-161503 on the reward-facilitating effect of cocaine.

Study 3: Effects of the intracranial injections of WAY-161503 on the reward-facilitating effect of cocaine

In the third study, four groups of animals (n=32) were used to evaluate the effects of the intracranial injection of WAY-161503 (0.15 and 0.3 μg/0.5 μl/side) or vehicle into specific regions of the mesolimbocortical dopaminergic system (VTA (n=8), NAc Core (n=8), NAc Shell (n=8), or mPFC (n=8) on the reward-facilitating effect of systemic cocaine (5 mg/kg, i.p.). The intracranial injection of WAY-161503 was given immediately following pre-drug rate–frequency function determination, followed by the cocaine injection. After a post-injection interval of 5 min, the rats were placed in the operant chamber, and the post-drug session began.

Data analysis and statistical treatment

The analysis was performed on two aspects of data obtained from the rate–frequency curve: the ICSS threshold and the maximum rate of responding or asymptote. The estimates were procured using the Gompertz sigmoid model (Coulombe and Miliaressis 1987):

$$f(x) = \alpha e^{-e^{b(x_i - x)}}$$

In this equalization, α represents the asymptote videlicet the maximum rate of responding, whereas X_i (X at inflection) represents the threshold frequency. The ICSS threshold is the pulse number producing 36.7% of the asymptotic rate. Parameter b represents an index of the slope whereas e is the base of natural logarithms.

The posttreatment threshold and asymptote values were expressed as percentage of pretreatment values. In the first study, the significance of the drug effect was statistically evaluated initially using one-way or two-way analyses of

variance (ANOVA)—depended on each case—with repeated measures followed, whenever appropriate, by paired samples *t* tests. In the second and third study, two-way ANOVA with repeated measures was performed to evaluate statistical significant interactions (5-HT_{2A} and 5-HT_{2C} agonists or antagonists—cocaine and antagonists—treatment (agonists and cocaine) and main effects. When the interaction was significant, we performed paired sample *t* tests or considered, whenever appropriate, Bonferroni's inequality approach, and the analysis of simple effects was tested in a

$$p = \frac{\text{The sum of } p\text{s for the main plus interaction effects}}{\text{Number of simple effects}}$$

For the last experiment of the second study (antagonist-agonist- cocaine), the six simple effects were tested at $p=0.025$. For the third study, the five simple effects were tested at $p=0.03$. The significance of simple effects was evaluated using repeated measures ANOVA followed, whenever appropriate, by correlated *t* test using Bonferroni's adjustment for multiple comparisons.

Histology

At the end of the experiment, the animals were given a lethal dose of sodium pentothal. The location of the terminal stimulation site was then marked according to the following procedure: a direct anodal current of 0.1 mA at 15-s duration was passed through the electrode tip. The animals were perfused intracardially with saline 0.9%, which was followed by a 50-cm^3 solution of potassium ferrocyanide (3%), potassium ferricyanide (3%), and trichloroacetic acid (0.5%) in 10% formalin. The brains were then removed and stored in 10% formalin for 3 days and 2 days in a 30% sucrose solution. Finally, the brains were sliced in a cryostat microtome, and the sections containing the electrode and the cannulae tracts were mounted on slides and stained with cresyl violet. Only the rats in which tracks from the electrode were verified to be located in the MFB at the level of the lateral hypothalamus, and tracks from the cannulae were verified to be located in the medial prefrontal cortex, the nucleus accumbens core/shell and the ventral tegmental area were included in this study (see Fig. 1).

Results

Behavioral studies

Study 1: Effects of systematically administered 5-HT_{2A} and 5-HT_{2C} ligands on brain stimulation reward

Experiment 1: Effects of systematically administered TCB-2 and R-96544 on brain stimulation reward

The changes in ICSS threshold and asymptotic rate of responding after systemic injections of TCB-2 are presented in Fig. 2a, b, respectively. One-way ANOVA with repeated measures demonstrated a significant drug effect ($F(3,18)=10.264$, $p<0.001$), on the ICSS threshold. Paired sample *t* tests revealed that TCB-2 significantly increased ICSS threshold at the dose of 0.3 mg/kg ($p<0.01$). The doses of 0.03 and 0.1 mg/kg did not produce significant effects ($p>0.05$). One-way ANOVA with repeated measures indicated no significant drug effect on the asymptotic rate of responding ($F(3,18)=2.809$, $p>0.05$).

The changes in ICSS threshold and asymptotic rate of responding after systemic injections of R-96544 are presented in Fig. 2c, d, respectively. One-way ANOVA with repeated measures indicated no significant drug effect on the ICSS threshold ($F(3,12)=0.797$, $p>0.05$), or on the asymptotic rate of responding ($F(3,12)=0.738$, $p>0.05$).

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of R-96544 and TCB-2 are presented in Fig. 2e, f, respectively. Two-way ANOVA with repeated measures demonstrated a significant interaction between the drugs ($F(1,6)=79.332$, $p<0.001$), on the ICSS threshold. Furthermore, paired sample *t* tests revealed that R-96544 successfully reversed the increased ICSS threshold produced by the administration of TCB-2 ($p<0.01$).

Experiment 2: Effects of systematically administered WAY-161503 and SB-242084 on brain stimulation reward

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of WAY-161503 are presented in Fig. 3a, b, respectively. One-way ANOVA with repeated measures demonstrated a significant drug effect ($F(3,18)=19.307$, $p<0.001$) on the ICSS threshold. Paired sample *t* tests revealed that WAY-161503 significantly increased ICSS threshold at the doses of 1 ($p<0.01$) and 3 mg/kg ($p<0.001$). One-way ANOVA with repeated measures indicated a significant drug effect on the asymptotic rate of responding ($F(3,18)=18.003$, $p<0.001$). Paired sample *t* tests revealed that the doses of 1 ($p<0.01$) and 3 mg/kg ($p<0.001$) produced a statistical significant decrease on the asymptotic rate of responding.

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of SB-242084 are presented in Fig. 3c, d, respectively. One-way ANOVA with repeated measures demonstrated no significant drug effect on the ICSS threshold ($F(3,21)=1.007$, $p>0.05$), or on the asymptotic rate of responding ($F(3,21)=0.895$, $p>0.05$).

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of SB-242084 and WAY-161503 are presented in Fig. 3e, f, respectively. Two-way ANOVA with repeated measures demonstrated a

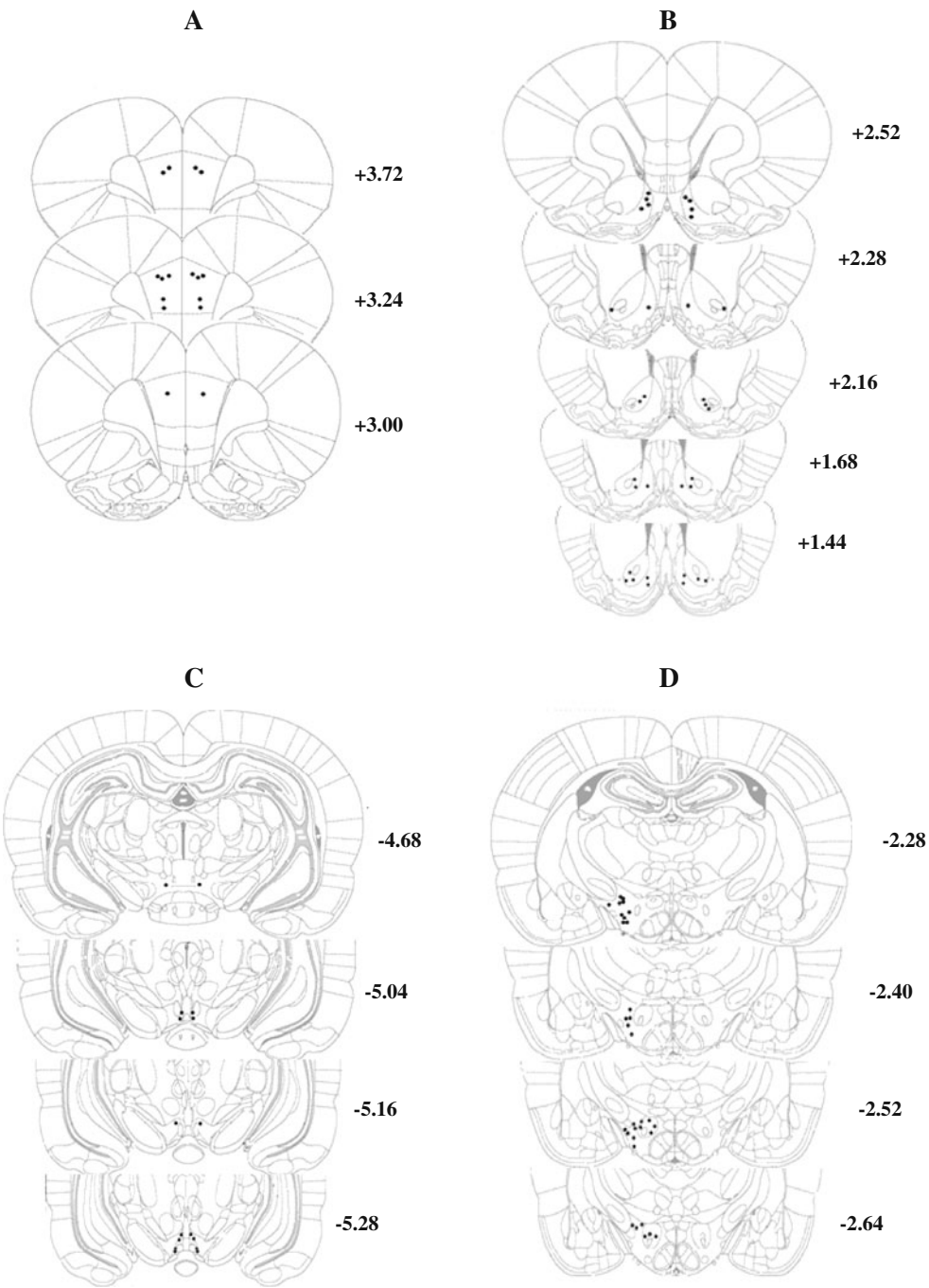

Fig. 1 Histological localization of injection cannula tips aimed at the mPFC (**a**), the NAC (**b**), and the VTA (**c**) and electrode tips aimed at the lateral hypothalamic level of MFB (**d**). The reconstructions are based on the stereotaxic atlas of Paxinos and Watson (2007). The *numbers* depicted next to each brain section indicate the distance (millimeter) from bregma

significant interaction between the drugs ($F(3,15)=1.118$, $p<0.001$) on the ICSS threshold. Furthermore, paired sample *t* tests revealed that SB-242084 successfully reversed the increased ICSS threshold produced by the administration of WAY-161503 ($p<0.001$). Two-way ANOVA with repeated measures demonstrated a significant interaction between the drugs ($F(3,15)=2.532$, $p<0.001$) on the asymptotic rate of responding. Furthermore, paired sample *t* tests revealed that SB-242084 successfully reversed the decreased asymptotic rate of responding produced by the administration of WAY-161503 ($p<0.01$).

Study 2: Effects of 5-HT_{2A} and 5-HT_{2C} receptor ligands on the reward-facilitating effect of cocaine

Experiment 1: Effects of TCB-2 and R-96544 on the reward-facilitating effect of cocaine

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of TCB-2 or its vehicle and cocaine or its vehicle are presented in Fig. 4a, b, respectively. Two-way ANOVA with repeated measures demonstrated a significant interaction of TCB-2 and cocaine

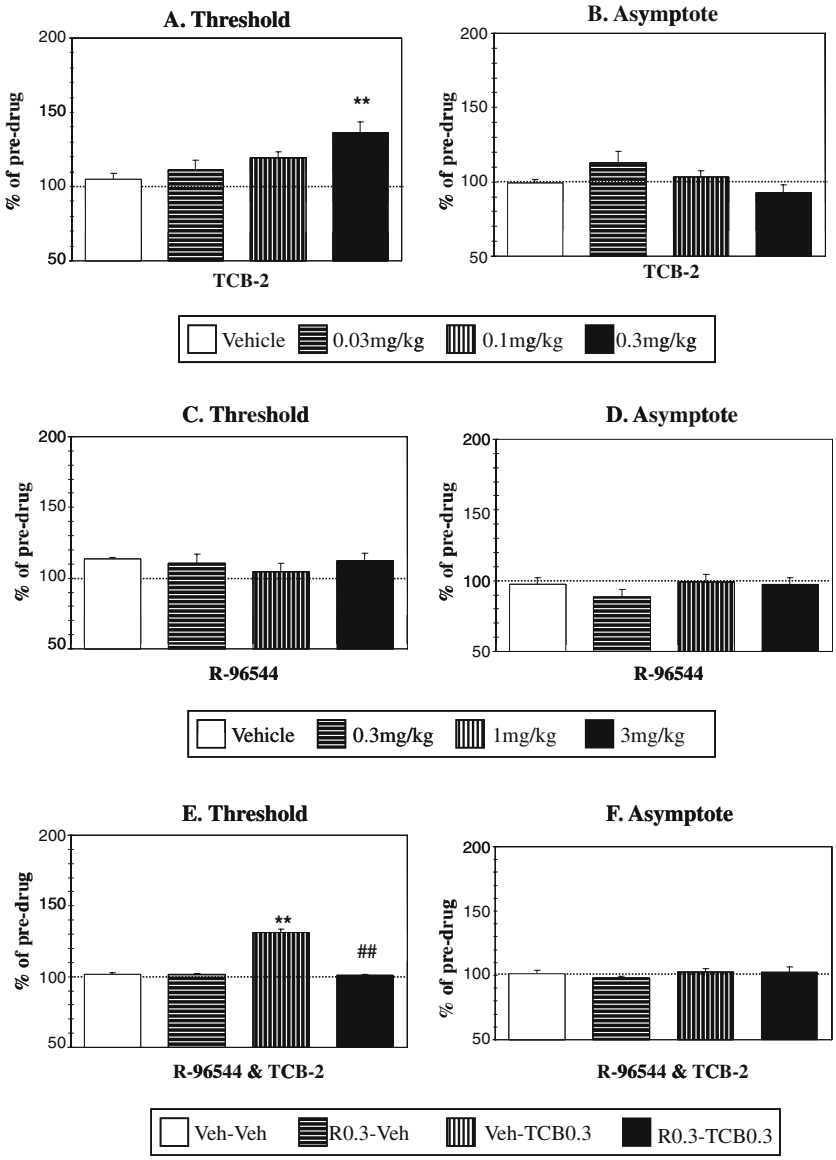

Fig. 2 Changes in ICSS threshold (**a**, **c**, **e**) and asymptotic rate (**b**, **d**, **f**) expressed as percentage of pre-drug values, following acute TCB-2, acute R-96544, and R-96544 (0.3 mg/kg, s.c.) combined with TCB-2 (0.3 mg/kg, i.p.) administration. *Vertical bars* represent the means ± SEM. The *asterisk* (*) signifies an ICSS threshold significantly different from the control group (*vehicle*), $^{**}p<0.01$. The *number sign* (#) signifies a statistically significant effect compared to the vehicle and TCB-2 group, $\#\#p<0.01$

(F(1,7)=13.245, $p<0.01$) on the ICSS threshold. Paired sample *t* tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). TCB-2 by itself did not affect ICSS threshold ($p>0.05$) and also failed to alter the reward-facilitating effect of cocaine ($p>0.05$). Two-way ANOVA with repeated measures indicated no statistical significant effects of TCB-2 (F(1,7)=2.978, $p>0.05$), cocaine (F(1,7)=2.328, $p>0.05$), or their interaction (F(1,7)=0.591, $p>0.05$) on the asymptotic rate of responding.

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of R-96544 or its vehicle and cocaine or its vehicle are presented in Fig. 4c, d, respectively. Two-way ANOVA with repeated measures demonstrated a significant effect of cocaine (F(1,7)=228.169, $p<0.001$) but no significant effect of R-96544 (F(1,7)=0.040, $p>0.05$) or significant interaction between these drugs (F(1,7)=0.799, $p>0.05$) on the ICSS threshold. Paired sample *t* tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). R-96544 by itself did not affect ICSS threshold ($p>0.05$) and also failed to alter the reward-facilitating effect of cocaine ($p>0.05$). Two-way ANOVA with repeated measures indicated no statistical significant effects of R-96544 (F(1,7)=0.423, $p>0.05$), cocaine (F(1,7)=0.263, $p>0.05$), or their interaction (F(1,7)=0.007, $p>0.05$) on the asymptotic rate of responding.

Experiment 2: Effects of WAY-161503 and SB-242084 on the reward-facilitating effect of cocaine

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of WAY-161503 or its vehicle and cocaine or its vehicle are presented in Fig. 5a, b, respectively. Two-way ANOVA with repeated measures demonstrated a significant interaction of WAY-161503 and

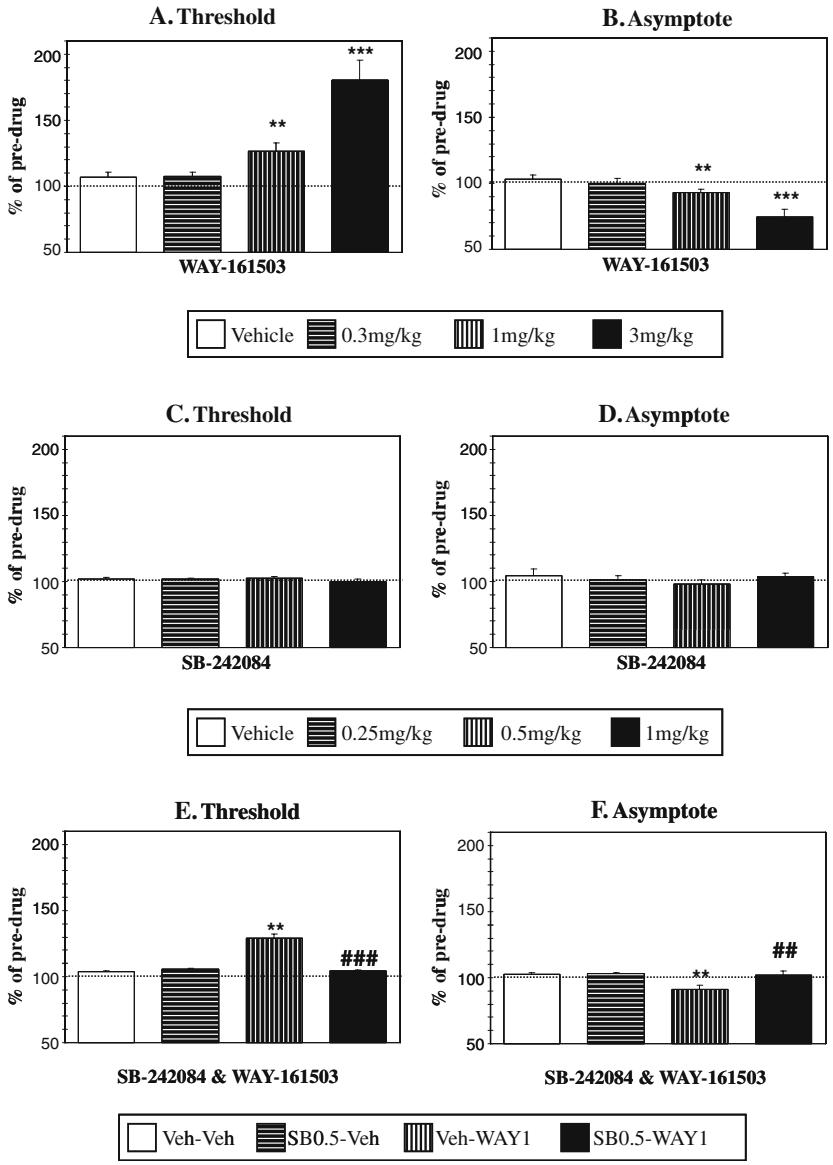

Fig. 3 Changes in ICSS threshold (**a**, **c**, **e**) and asymptotic rate (**b**, **d**, **f**) expressed as percentage of pre-drug values, following acute WAY-161503, acute SB-242084, and SB-242084 (0.5 mg/kg, i.p.) combined with WAY-161503 (1 mg/kg, s.c.) administration. *Vertical bars* represent the means±SEM. The *asterisk* (*) signifies an ICSS threshold or asymptote value significantly different from the control group (*vehicle*), $^{**}p<0.01$; $^{***}p<0.001$. The *number sign* (#) signifies a statistically significant effect compared to the vehicle and WAY-161503 group, $\#\#p<0.01$; $\#\#\#p<0.001$

cocaine ($F(1,7)=20.463$, $p<0.001$) on the ICSS threshold. Paired sample *t* tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). Furthermore, WAY-161503 by itself did not affect ICSS threshold ($p>0.05$), while completely reversed the reward-facilitating effect of cocaine ($p<0.001$). Two-way ANOVA with repeated measures indicated no statistical significant effects of WAY-161503 ($F(1,7)=2.515$, $p>0.05$), cocaine ($F(1,7)=0.001$, $p>0.05$), or their interaction ($F(1,7)=2.349$, $p>0.05$) on the asymptotic rate of responding.

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of SB-242084 or its vehicle and cocaine or its vehicle are presented in Fig. 5c, d, respectively. Two-way ANOVA with repeated measures demonstrated a significant interaction of SB-242084 and cocaine ($F(1,7)=20.463$, $p<0.001$) on the ICSS threshold. Paired sample *t* tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). SB-242084 by itself did not affect ICSS threshold ($p>0.05$), while it appeared to act synergistically with cocaine, decreasing further the ICSS threshold ($p<0.001$). Two-way ANOVA with repeated measures indicated no statistical significant effects of SB-242084 ($F(1,7)=1.502$, $p>0.05$), cocaine ($F(1,7)=0.784$, $p>0.05$), or their interaction ($F(1,7)=5.768$, $p>0.05$) on the asymptotic rate of responding.

The changes in ICSS threshold and asymptotic rate of responding after systemic injection of SB-242084, WAY-161503, and cocaine or their vehicle are presented in Fig. 5e, f, respectively. Two-way ANOVA with repeated measures showed a statistical significant interaction between the three drugs ($F(1,7)=83.901$, $p<0.001$) on the ICSS threshold. Repeated measures on the simple effect of

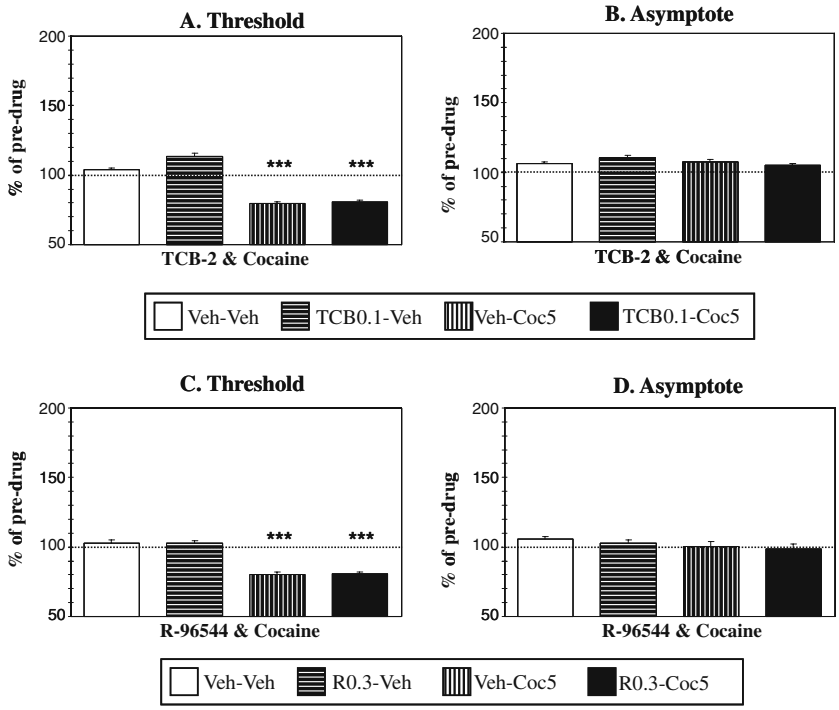

Fig. 4 Changes in ICSS threshold (**a**, **c**) and asymptotic rate (**b**, **d**) expressed as percentage of pre-drug values, following acute administration of TCB-2 (0.1 mg/kg, i.p.) and cocaine (5 mg/kg, i.p.) and R-96544 (0.3 mg/kg, s.c.) and cocaine (5 mg/kg, i.p.). *Vertical bars* represent the means±SEM. The *asterisk* (*) signifies an ICSS threshold significantly different from the control group (vehicle and vehicle), ***$p<0.001$

SB-242084 (0.5 mg/kg) showed a statistical significant effect ($F(3,21)=90.633$, $p<0.001$). Furthermore, paired samples *t* test using Bonferroni's adjustment for multiple comparisons showed that SB-242084 completely reversed the action of WAY-161503 on the reward-facilitating effect of cocaine ($p<0.001$).

Study 3: Effects of the intracranial injections of WAY-161503 on the reward-facilitating effect of cocaine

Experiment 1: Intracranial injections into the medial prefrontal cortex

The changes in ICSS threshold and asymptotic rate of responding after intra-mPFC injection of WAY-161503 or its vehicle and systemic injection of cocaine or its vehicle are presented in Fig. 6a, b, respectively. Two-way ANOVA with repeated measures showed a statistical significant interaction of WAY-161503 and cocaine ($F(2,14)=76.299$, $p<0.001$) on the ICSS threshold. Paired sample *t* tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). Repeated measures on the simple effect of cocaine's vehicle showed no statistical significant effect of WAY-161503 ($F(2,14)=1.551$, $p>0.03$). Repeated measures on the simple effect of cocaine (5 mg/kg) showed a statistical significant effect of WAY-161503 ($F(2,14)=91.453$, $p<0.001$). Paired samples *t* test using Bonferroni's adjustment for multiple comparisons reveal that acute administration of WAY-161503 at the dose of 0.3 μg/side blocked the reward-facilitating effect of cocaine on ICSS ($p<0.001$). On the contrary, WAY-161503 at the dose of 0.15 μg/side did not block this effect ($p>0.05$). Two-way ANOVA with repeated measures indicated no statistical significant effect of WAY-161503 ($F(2,14)=0.656$, $p>0.05$), cocaine ($F(1,7)=5.763$, $p>0.05$), or their interaction ($F(2,14)=2.511$, $p>0.05$) on the asymptotic rate of responding.

Experiment 2: Intracranial injections into the core of the nucleus accumbens

The changes in ICSS threshold and asymptotic rate of responding after intra-NAC core injection of WAY-161503 or its vehicle and systemic injection of cocaine or its vehicle are presented in Fig. 6c, d, respectively. Two-way ANOVA with repeated measures showed a statistical significant interaction of WAY-161503 and cocaine ($F(2,14)=40.179$, $p<0.001$) on the ICSS threshold. Paired sample *t* tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). Repeated measures on the simple effect of cocaine's vehicle showed no statistical significant effect of WAY-161503 ($F(2,14)=1.304$, $p>0.03$). Repeated measures on the simple effect of cocaine (5 mg/kg) showed a statistical significant effect of WAY-161503 ($F(2,14)=89.005$, $p<0.001$). Paired samples *t* test using Bonferroni's adjustment for multiple comparisons reveal that acute administration of WAY-161503 at the dose of 0.3 μg/side blocked the reward-facilitating effect of cocaine on ICSS ($p<0.001$). On the contrary, WAY-161503 at the dose of 0.15 μg/side did not block this effect ($p>$

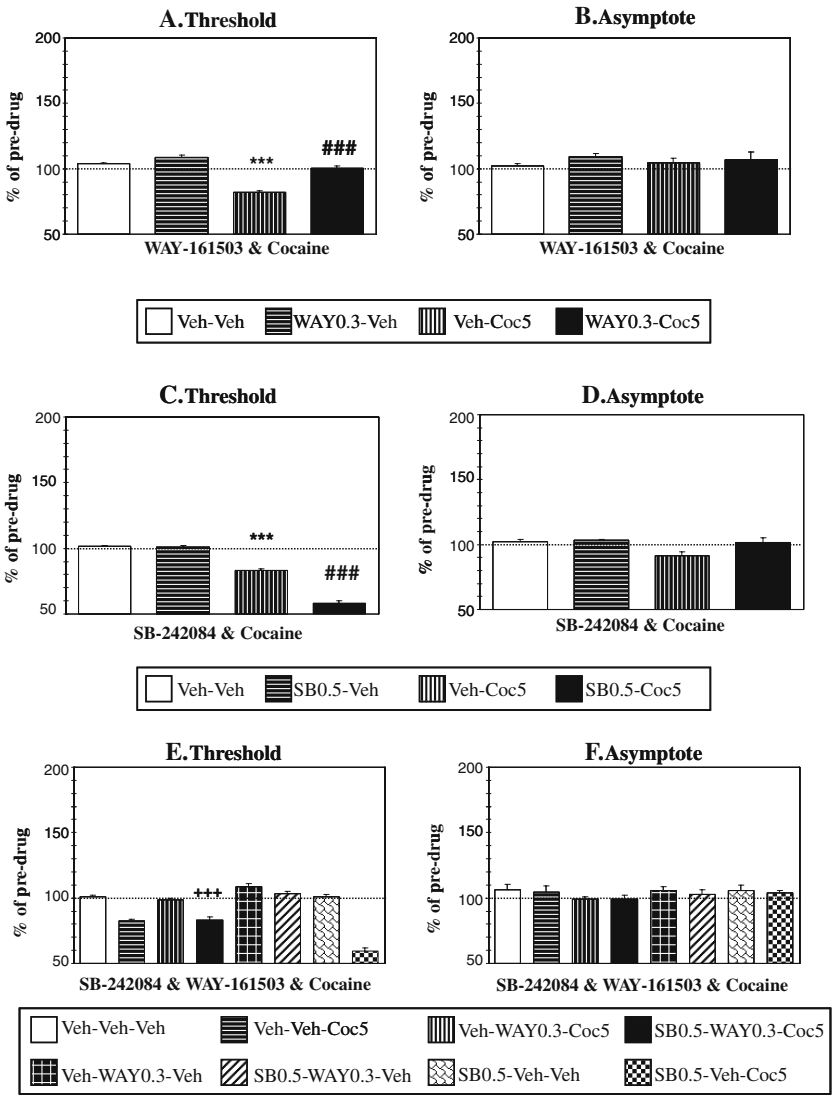

Fig. 5 Changes in ICSS threshold (**a**, **c**, **e**) and asymptotic rate (**b**, **d**, **f**) expressed as percentage of pre-drug values, following acute administration of WAY-161503 (0.3 mg/kg, s.c.) and cocaine (5 mg/kg), SB-242084 (0.5 mg/kg, i.p.), and cocaine and SB-242084 and WAY-161503 and cocaine. *Vertical bars* represent the means±SEM. The *asterisk* (*) signifies an ICSS threshold significantly different from the control group (vehicle and vehicle), ***$p<0.001$. The *number sign* (#) signifies a statistically significant effect compared to the vehicle and cocaine group (###$p<0.001$). The *plus sign* (+) signifies a statistically significant effect compared to the vehicle and WAY-161503 and cocaine group, +++$p<0.001$

0.05). Two-way ANOVA with repeated measures indicated no statistical significant effect of WAY-161503 ($F(2,14)=3.362$, $p>0.05$), cocaine ($F(1,7)=2.771$, $p>0.05$), or their interaction ($F(2,14)=0.111$, $p>0.05$) on the asymptotic rate of responding.

Experiment 3: Intracranial injections into the shell of the nucleus accumbens

The changes in ICSS threshold and asymptotic rate of responding after intra-NAC shell injection of WAY-161503 or its vehicle and systemic injection of cocaine or its vehicle are presented in Fig. 6e, f, respectively. Two-way ANOVA with repeated measures showed a statistical significant interaction of WAY-161503 and cocaine ($F(2,14)=83.299$, $p<0.001$) on the ICSS threshold. Paired sample t tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). Repeated measures on the simple effect of cocaine's vehicle showed no statistical significant effect of WAY-161503 ($F(2,14)=0.231$, $p>0.03$). Repeated measures on the simple effect of cocaine (5 mg/kg) showed a statistical significant effect of WAY-161503 ($F(2,14)=85.236$, $p<0.001$). Paired samples t test using Bonferroni's adjustment for multiple comparisons reveal that acute administration of WAY-161503 at the dose of 0.3 μg/side blocked the reward-facilitating effect of cocaine on ICSS ($p<0.001$). On the contrary, WAY-161503 at the dose of 0.15 μg/side did not block this effect ($p>0.05$). Two-way ANOVA with repeated measures indicated no statistical significant effect of WAY-161503 ($F(2,14)=0.848$, $p>0.05$), cocaine ($F(1,7)=7.954$, $p>0.05$), or their interaction ($F(2,14)=1.614$, $p>0.05$) on the asymptotic rate of responding.

Experiment 4: Intracranial injections into the ventral tegmental area

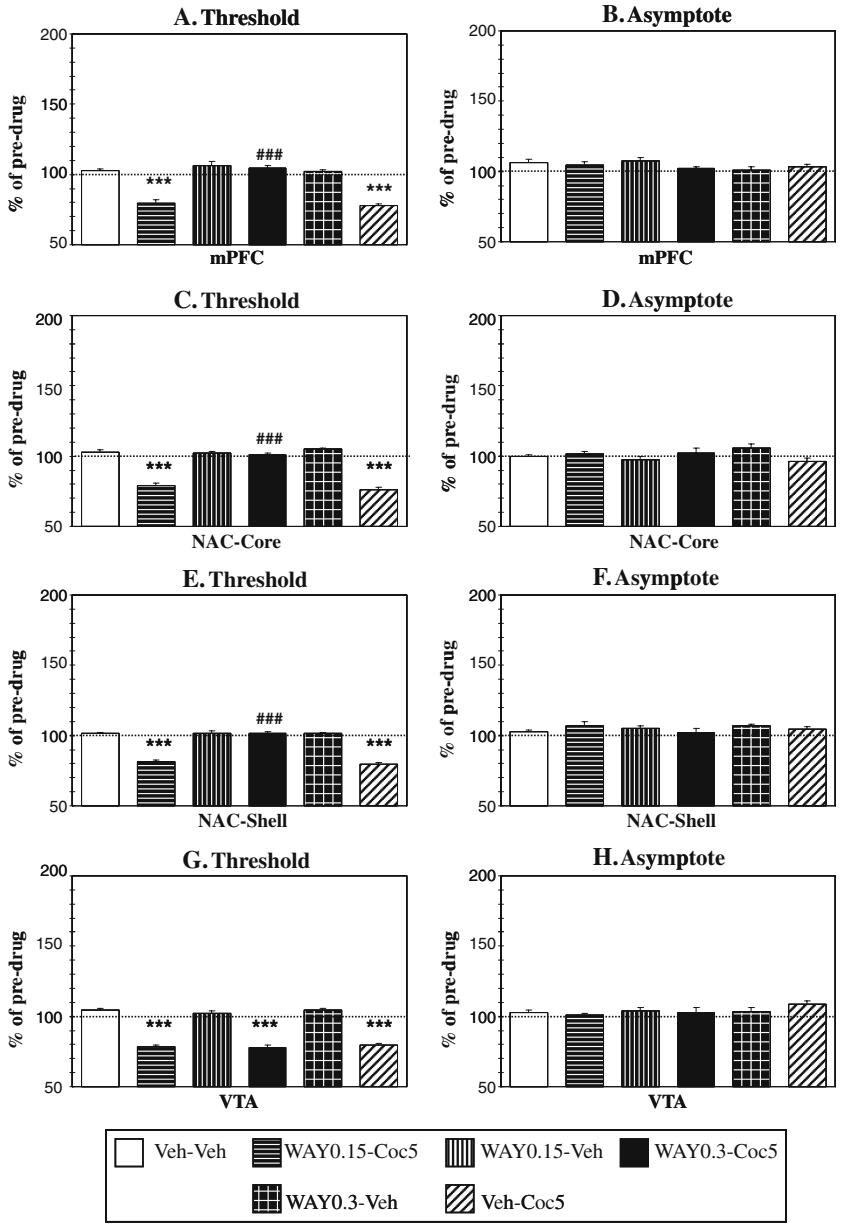

Fig. 6 Changes in ICSS threshold (**a**, **c**, **e**, **g**) and asymptotic rate (**b**, **d**, **f**, **h**) expressed as percentage of pre-drug values, following intra-mPFC (**a**, **b**), intra-NAc (**c**, **d**, **e**, **f**) and intra-VTA (**g**, **h**) injection of WAY-161503 (0.15 and 0.3 μg/side) followed by acute and systemic administration of cocaine (5 mg/kg, i.p.) *Vertical bars* represent the means±SEM. The *asterisk* (*) signifies an ICSS threshold significantly different from the control group (vehicle and vehicle), ***$p<0.001$. The *number sign* (#) signifies a statistically significant effect compared to the vehicle and cocaine group, ### $p<0.001$

The changes in ICSS threshold and asymptotic rate of responding after intra-VTA injection of WAY-161503 or its vehicle and systemic injection of cocaine or its vehicle are presented in Fig. 6g, h, respectively. Two-way ANOVA with repeated measures showed a statistical significant interaction of WAY-161503 and cocaine ($F(2,14)=42.199$, $p<0.001$) on the ICSS threshold. Paired sample t tests revealed that cocaine significantly decreased the ICSS threshold ($p<0.001$). Repeated measures on the simple effect of cocaine's vehicle showed no statistical significant effect of WAY-161503 ($F(2,14)=3.504$, $p>0.03$). Repeated measures on the simple effect of cocaine (5 mg/kg) also showed no statistical significant effect of WAY-161503 ($F(2,14)=3.504$, $p>0.03$). Two-way ANOVA with repeated measures indicated no statistical significant effect of WAY-161503 ($F(2,14)=2,477$, $p>0.05$), cocaine ($F(1,7)=0.084$, $p>0.05$), or their interaction ($F(2,14)=1.536$, $p>0.05$) on the asymptotic rate of responding.

Figures 7 and 8 depict rate–frequency functions from representative animals obtained before and after administration of various treatment combinations of 5-HT_{2A} and 5-HT_{2C} ligands and cocaine. As indicated, the vehicle + cocaine treatment produced a parallel curve shift to the left, indicating a clear increase in the rewarding efficacy of the stimulation. On the other hand, the systemic, intra-mPFC, intra-NACcore, or intra-NACshell WAY-161503+cocaine treatment produced no effect on ICSS, blocking the reward-facilitating effect of cocaine.

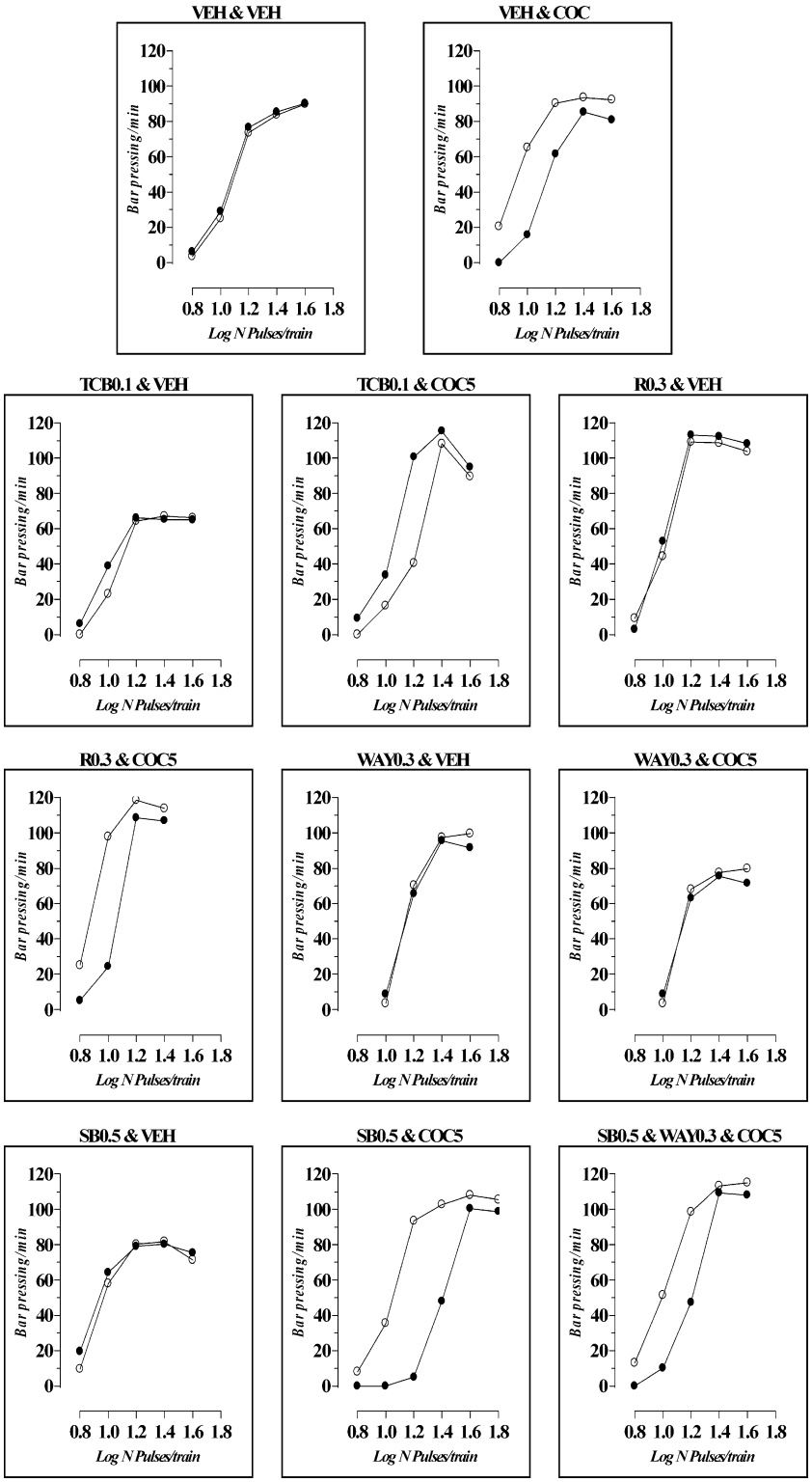

Fig. 7 Rate–frequency functions (rate of lever pressing as a function of stimulation frequency) taken from representative animals for each drug treatment with systemic injections. *Each plot* represents data from a single animal under pre-drug and drug conditions. Rate–frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing. *Filled circle* pre-injection; *empty circle* post-injection

Discussion

The present studies were undertaken to examine the role of 5-HT_{2A} and 5-HT_{2C} receptors in brain stimulation reward and the reward-facilitating effect of cocaine and localize the neural substrates within the mesolimbocortical dopaminergic system that are responsible for these effects. The results revealed that systemic injection of the 5-HT_{2A} receptor agonist TCB-2 or the 5-HT_{2C} receptor agonist WAY-161501 increase ICSS thresholds. However, only the 5-

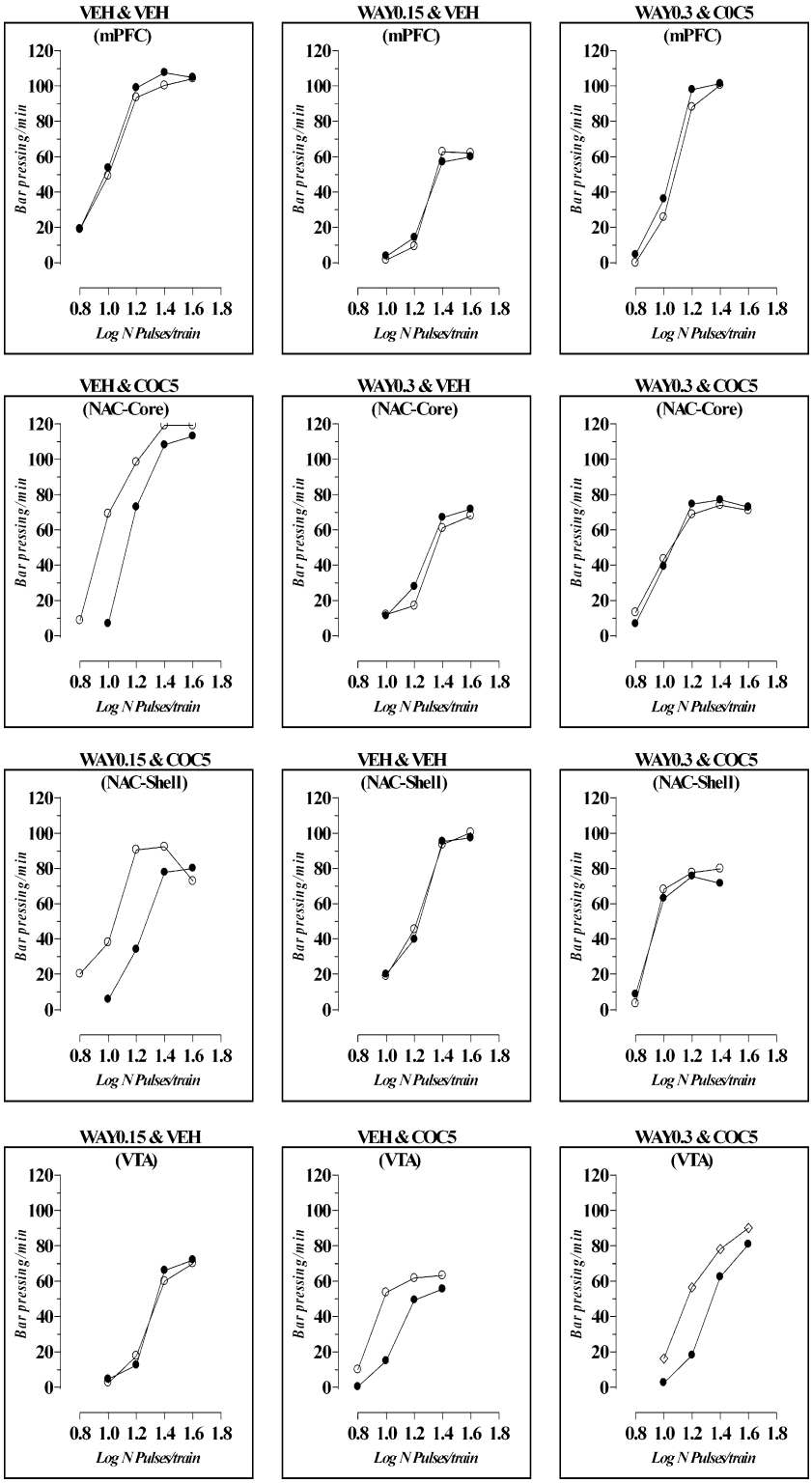

Fig. 8 Rate–frequency functions (rate of lever pressing as a function of stimulation frequency) for intracranial injections taken from representative animals for each brain region. *Each plot* represents data from a single animal under pre-drug and drug conditions. Rate–frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing. *Filled circle* pre-injection; *empty circle* post-injection

HT_{2C} receptor agonist WAY-161501 was able to counteract the reward-facilitating effect of cocaine. Moreover, the present experiments indicate that the shell and core of the NAC and the mPFC, but not the VTA, are the critical regions for this serotoninergic modulation of the reward-facilitating effect of cocaine. Specifically, it was found that microinjection of the 5-HT_{2C} receptor agonist WAY-161501 into the major terminal regions of the mesolimbocortical dopeminergic system, i.e., the shell and core of the NAC and the mPFC, blocks the reward-facilitating effect of

cocaine, while microinjection of WAY-161501 into the VTA, i.e., the somatodentritic region of this circuit, has no effect.

Lateral hypothalamus is the most commonly used site in self-stimulation research. However, since differences between LH and VTA ICSS have been reported in the literature (Singh et al. 1996), the herein reported effects of the 5-HT_{2A} and 5-HT_{2C} receptor agonists and antagonists on ICSS thresholds in lateral hypothalamic sites should not be generalized to other brain regions, and more extensive investigation of other brain regions within the MFB, such as the NAC and the VTA, is needed.

Our first study demonstrated that the selective 5-HT_{2A} receptor antagonist R-96544 and 5-HT_{2C} receptor antagonist SB-242084 did not affect brain stimulation reward. However, both the 5-HT_{2A} receptor agonist TCB-2 and the 5-HT_{2C} receptor agonist WAY-161503 significantly increased ICSS thresholds. The anhedonic effect of TCB-2 and WAY-161503 was completely abolished by pretreatment with the 5-HT_{2A} and 5-HT_{2C} receptor antagonists R-96544 and SB-242084, respectively. In the past few years, considerable attention has been given to the relative importance of different 5-HT_2 receptor subtypes in mediating reward-related behaviors. However, only a few studies have examined the effect of selective 5-HT_{2A} and 5-HT_{2C} receptor agonists and antagonists on brain stimulation reward. Our result with R-96544 is in accordance with a study by Benaliouad and colleagues (2007), in which the selective 5-HT_{2A} receptor antagonist M-100907 did not affect brain stimulation reward. Recently, it was demonstrated that the selective 5-HT_{2C} receptor agonist WAY-161503 significantly increased ICSS thresholds, whereas the selective 5-HT_{2C} receptor antagonist SB-242084 did not affect brain stimulation reward (Hayes et al. 2009a). Our results are consistent with the latter findings but contradict the prevailing view that 5-HT_{2A} and 5-HT_{2C} receptor stimulation induces opposite behavioral, neurophysiological, and neurochemical effects (Bubar and Cunningham 2006; Muller and Huston 2006). Thus, the increase in ICSS thresholds following systemic administration of the selective 5-HT_{2A} and 5-HT_{2C} receptor agonists indicates that both 5-HT_{2A} and 5-HT_{2C} receptor stimulation plays an inhibitory role in ICSS. Because of the relatively recent development of TCB-2 as a selective ligand for the 5-HT_{2A} receptors, there are no other behavioral studies in the literature reporting its effect on brain reward. Nonetheless, our result concerning the 5-HT_{2A} receptors agrees with the notion that all known psychoactive drugs that act as 5-HT_{2A} receptor agonists (i.e., lysergic acid diethylamide, mescaline, psilocybin), although affecting perception, cognition, and mood in humans, do not exhibit reinforcing properties in experimental animals.

TCB-2 did not affect maximal rates of responding in any of the doses tested. On the other hand, the highest dose of WAY-161503 decreased the maximal rates of responding. This is in agreement with studies showing an inhibitory effect of 5-HT_{2C} receptor agonists on motor activity (Filip et al. 2004, 2006; Fletcher et al. 2002a; Hayes et al. 2009b; McCreary and Cunningham 1999). However, there is strong evidence that the presently used ICSS paradigm provides reward threshold estimates that are unaffected by performance effects of drug treatments or other experimental manipulations (Edmonds and Gallistel 1974; Miliaressis and Rompre 1987). This is also evident in the present study, where the increases in ICSS thresholds produced by TCB-2 were not accompanied by significant changes in asymptotic rates of responding.

Another major finding of the present study is that acute administration of WAY-161503 in a dose that by itself was ineffective in altering brain stimulation reward thresholds, but not TCB-2, counteracts the reward-facilitating effect of systemic cocaine. Cocaine (5 mg/kg) produced a significant reduction in ICSS threshold, without altering maximal rates of responding. The presently observed effects of cocaine in the reinforcing efficacy of brain stimulation are in agreement with other studies, which have shown that cocaine decreases brain stimulation reward threshold (Gallo et al. 2010; Maldonado-Irizarry et al. 1994; Matthews et al. 1996; Panagis et al. 2000; Panagis and Spyraki 1996; Ranaldi et al. 1997; Vlachou et al. 2003, 2008). WAY-161503 (0.3 mg/kg) attenuated the reward-facilitating action of cocaine, an effect which was reversed by pretreatment with the selective 5-HT_{2C} receptor antagonist SB-242084 (0.5 mg/kg), further suggesting a 5-HT_{2C} receptor-mediated effect. Moreover, the systemic administration of SB-242084 potentiated the reward-facilitating effect of cocaine. This finding is consistent with other behavioral studies (Fletcher et al. 2002a) and clearly suggests that 5-HT_{2C} rather than 5-HT_{2A} receptor blockade potentiates the reward-facilitating effect of cocaine.

Overall, the present results are in agreement with previous studies, which have shown that 5-HT_{2A} and 5-HT_{2C} receptors exert opposing effects on the behavioral and neurochemical effects of cocaine. Several studies using systemic administration of the 5-HT_{2A} receptor agonist DOI and the selective 5-HT_{2A} receptor antagonist M-100907 indicate that 5-HT_{2A} receptor stimulation facilitates cocaine-induced hyperlocomotion and sensitization (Filip et al. 2004; Fletcher et al. 2002a). Although several studies demonstrate that 5-HT_{2A} receptors attenuate both cocaine-primed and cue-evoked reinstatement of cocaine self-administration (Burmeister et al. 2004; Filip 2005; Fletcher et al. 2002a), there is no evidence for a direct role of this receptor in cocaine self-administration (Fletcher et al. 2002a; Howell and Byrd 1995), which when corroborated

by our data might indicate that 5-HT_{2A} receptors are not involved in the direct reinforcing/rewarding effects of cocaine. On the other hand, there is ample evidence for an inhibitory role of 5-HT_{2C} receptors in cocaine-induced hyperlocomotion, self-administration, and cocaine-primed or cue-evoked reinstatement of self-administration (Filip et al. 2004; Fletcher et al. 2002a; Grottick et al. 2000; Rocha et al. 2002). Indeed, the selective 5-HT_{2C} receptor antagonist SB-242084 increased (Fletcher et al. 2002a), whereas the selective 5-HT_{2C} receptor agonist Ro60-0175 reduced rates of cocaine self-administration (Fletcher et al. 2004; Grottick et al. 2000). Similar results were also observed in studies with other drugs of abuse, since 5-HT_{2C} receptor stimulation reduced responding for ethanol (Tomkins et al. 2002) and nicotine (Grottick et al. 2001) self-administration. Interestingly, there have been other reports in the literature indicating that 5-HT_{2A} and 5-HT_{2C} receptors can produce opposite behavioral effects. For example, in the signal attenuation rat model of obsessive–compulsive disorder, a selective 5-HT_{2C} antagonist, but not a selective 5-HT_{2A} antagonist, produces anti-compulsive effects (Flaisher-Grinberg et al. 2008). Similarly, selective 5-HT_{2A} and 5-HT_{2C} antagonists show dissociable effects in the five-choice serial reaction test and the serial spatial reversal learning task in rats (Boulougouris et al. 2008; Boulougouris and Robbins 2010; Robinson et al. 2008). These data, coupled with our demonstration that WAY-161503 blocks the reward-facilitating effect of cocaine on ICSS, suggest that 5-HT_{2C} receptor agonists may reduce the reinforcing efficacy of cocaine.

Pharmacological studies have shown that both 5-HT_{2A} and 5-HT_{2C} receptors can modulate dopaminergic neurotransmission within the mesolimbocortical dopaminergic system (Alex and Pehek 2007; Fink and Gothert 2007). Thus, the mechanism by which 5-HT_{2C} receptors control the reward-facilitating effect of cocaine is likely mediated through modulation of dopaminergic neurotransmission. A series of studies have shown that selective 5-HT_{2C} receptor antagonists enhance mesolimbocortical dopaminergic function (i.e., increase basal firing rate of VTA dopaminergic neurons and dopamine release in the NAC), whereas selective 5-HT_{2C} receptor agonists have the opposite effect (Di Matteo et al. 2001, 2002). Similar to the effects observed upon basal dopamine release, it has been demonstrated that intra-VTA injection of the 5-HT_{2C} receptor agonists Ro60-0175 attenuates cocaine-induced dopamine release in the NAC (Navailles et al. 2008). Moreover, activation of 5-HT_{2C} receptors inhibits morphine-induced dopamine release in the NAC (Willins and Meltzer 1998). To the contrary, 5-HT_{2C} receptor antagonists enhanced cocaine-induced dopamine release in the NAC (De Deurwaerdere et al. 2004). These data suggest that 5-HT_{2C} receptors play important inhibitory role in modulating the effect of cocaine and other drugs of abuse on dopamine output. Thus, we speculate that the herein reported inhibitory action of WAY-161503 on the reward-facilitating effect of cocaine must be mediated by the aforementioned dopaminergic mechanisms of action. The same mechanisms may explain the finding that the systemic administration of SB-242084 potentiated the reward-facilitating effect of cocaine.

Another major finding of the present study is that intra-mPFC and intra-NAC administration of the 5-HT_{2C} receptor agonist WAY-161503 attenuated the reward-facilitating effect of cocaine. In contrast, intra-VTA administration of WAY-161503 had no effect. Several studies have shown that the effects of local stimulation of 5-HT_{2C} receptors on cocaine-related behaviors and actions differ considerably, depending on the site of injection. Furthermore, the effects of local pharmacological manipulation of 5-HT_{2C} receptors on the behavioral responses to cocaine have been inconsistent (Muller and Huston 2006). For example, intra-NAC injection of 5-HT_{2C} receptor agonists facilitates cocaine-induced hyperlocomotion (Filip and Cunningham 2002; McMahon and Cunningham 2001), whereas intra-mPFC or intra-VTA injection of the same compounds block cocaine-induced hyperlocomotion (Filip and Cunningham 2003; Fletcher et al. 2004) and attenuate cocaine self-administration (Fletcher et al. 2004). In our study, WAY-161503 (0.15 or 0.3 μg/μl/side) had no effect on lateral hypothalamic self-stimulation when microinjected into the VTA, the core, and the shell of the NAC or the mPFC. These data are consistent with a recent study by Hayes and colleagues (2009a), in which intra-NAC WAY-161503 did not affect VTA ICSS. The failure of intra-VTA injection of WAY-161503 to block the reward-facilitating effect of cocaine may be related to the reported excitatory action of the 5-HT_{2C} receptors located on the dopaminergic neurons of the VTA (Bubar and Cunningham 2007), which would lead to an excitation of dopaminergic activity, counteracting the indirect inhibition of the neuronal firing of dopaminergic neurons via GABAergic neurons of the VTA. More likely, the finding that stimulation of VTA 5-HT_{2C} receptors by systemic WAY-161503 counteracts the reward-facilitating effect of cocaine suggests that the reported effect may be related to modulatory actions of the 5-HT_{2C} receptors in other brain regions, such as the NAC and the mPFC. Indeed, there is evidence that 5-HT_{2C} receptors within the NAC exert an inhibitory control over cocaine-induced dopamine release in this terminal region (Navailles et al. 2008). In line with this evidence, in the present study intra-NAC injection of WAY-161503 counteracted the reward-facilitating effect of cocaine. With regard to the results from intra-mPFC studies, the inhibitory effect of WAY-161503 on the reward-facilitating action of cocaine may be related to the stimulation of 5-HT_{2C} receptors found

within GABAergic interneurons, which, in turn, would be expected to reduce the excitatory output to medium spiny neurons in the NAC and/or the dopaminergic neurons in the VTA. Alternatively, 5-HT_{2C} receptors within the NAC and the mPFC may inhibit the reward-facilitating effect of cocaine independently from dopaminergic mechanisms within the NAC, by controlling dopaminergic neurotransmission downstream from dopaminergic neurons (Navailles et al. 2004, 2008).

In conclusion, the inhibitory action of 5-HT_{2C} receptors after systemic, intra-NAC, and intra-mPFC administration presented in this study is important to understand the brain mechanisms involved in the reward-facilitating effect of cocaine and the processes involved in cocaine addiction. The present findings suggest that 5-HT_{2C} receptors clearly play a modulatory role in cocaine's reinforcing action. Based upon the present findings, we speculate that 5-HT_{2C} receptor agonists could be further tested as a potential treatment for psychostimulant addiction.

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